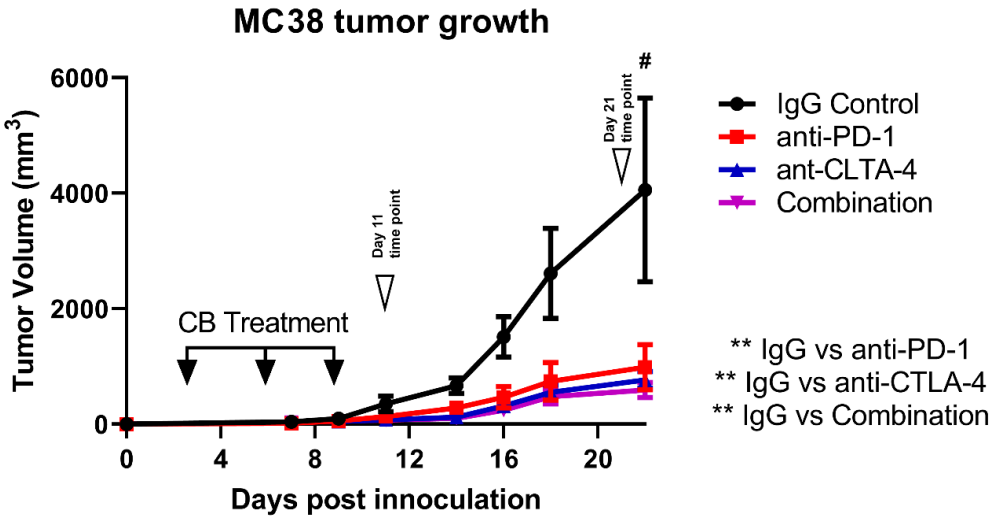
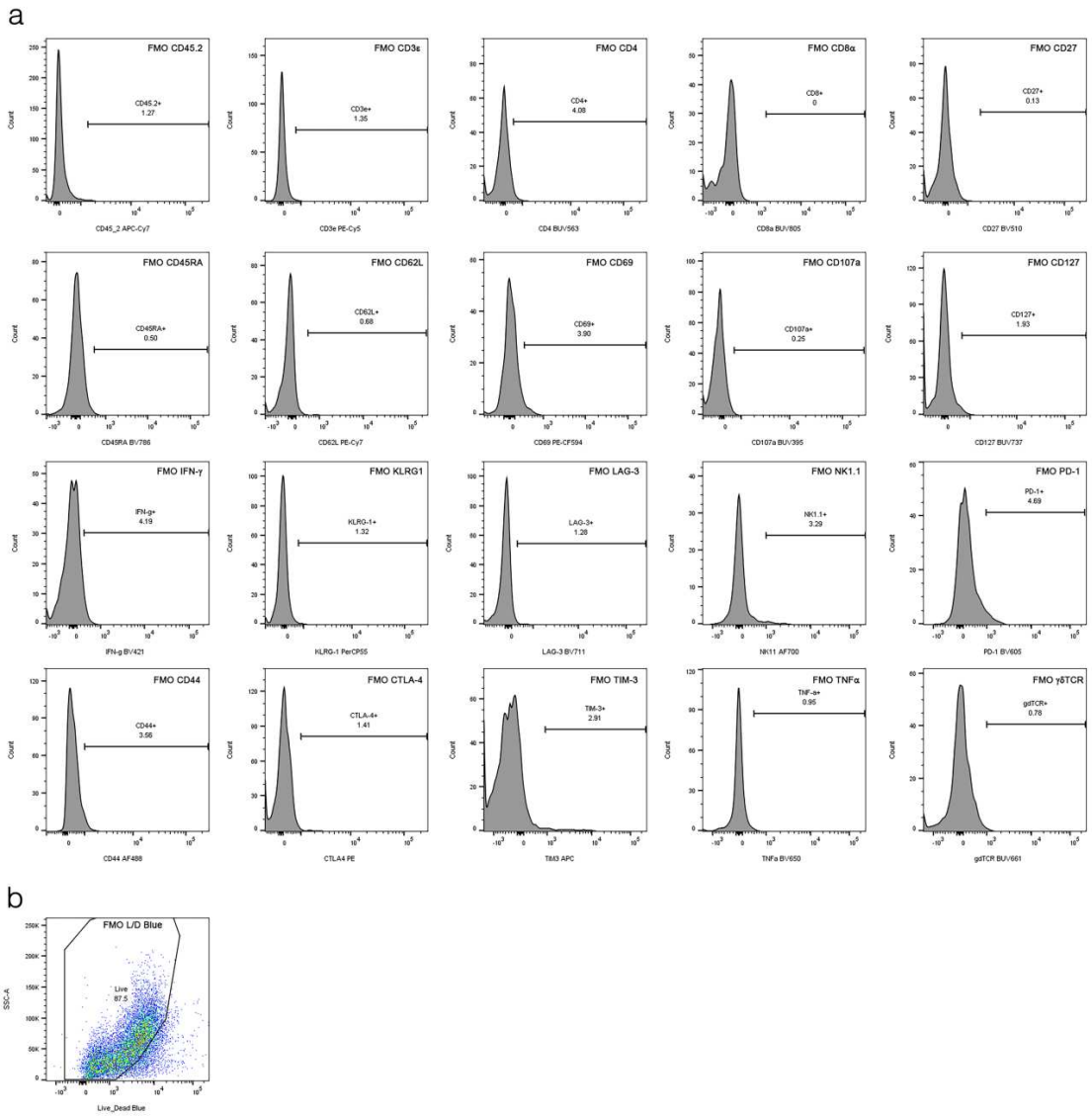
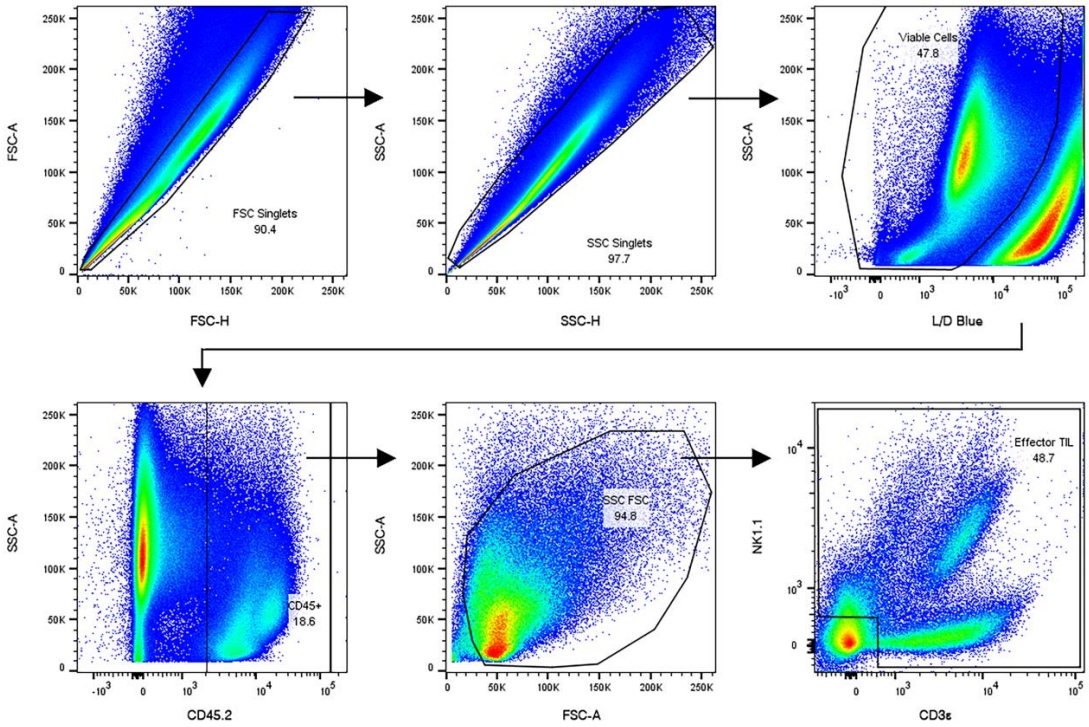
Supplementary Information



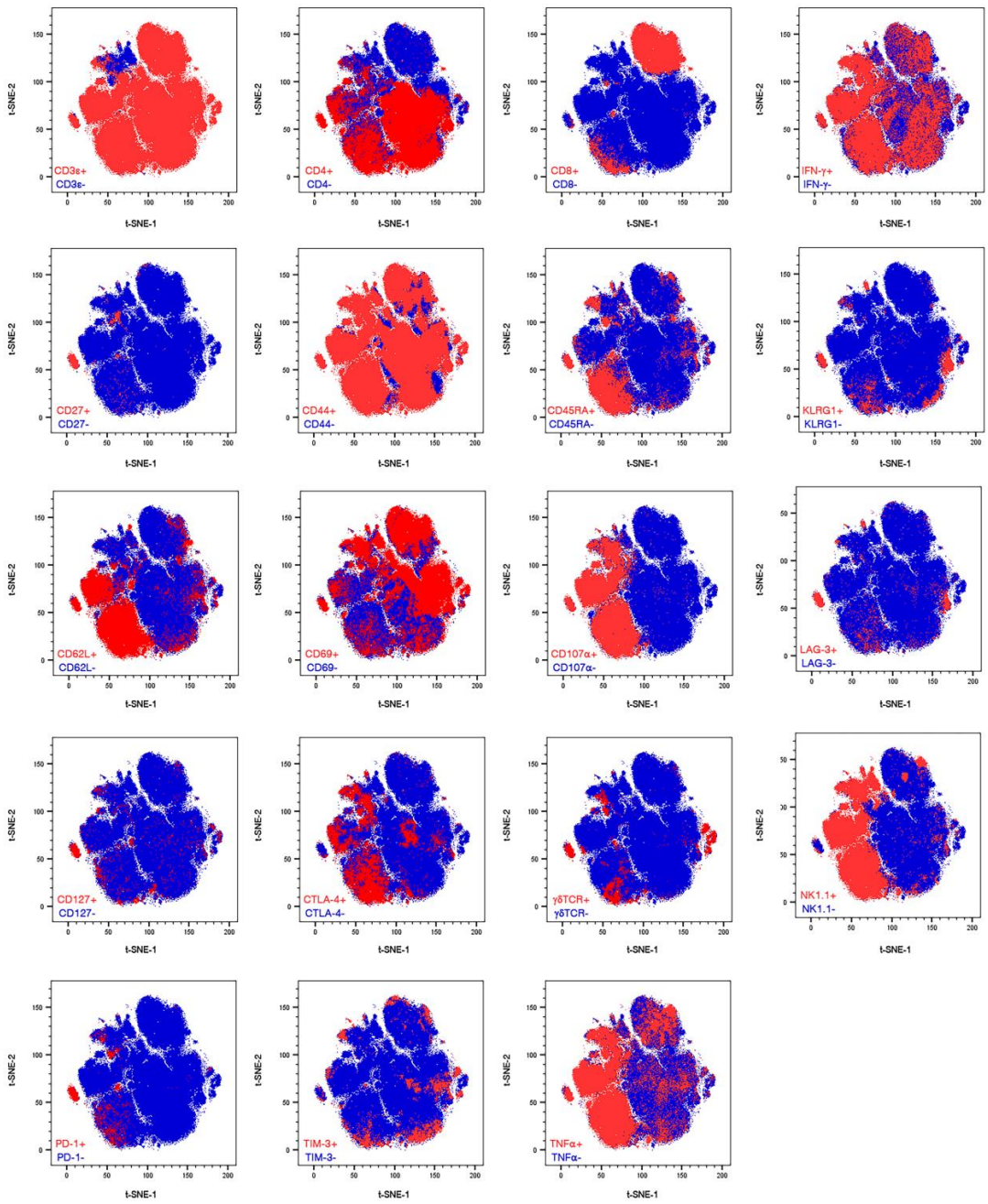
Supplemental Figure 1: The effect of checkpoint blockade on MC38 tumor growth. C57BL/6 mice were inoculated subcutaneously in the upper back with $1.0x10^6$ MC38 cells in sterile PBS (100 μL) and were then treated with intraperitoneal injections with control IgG, anti-PD-1, anti-CTLA-4, or a combination of anti-PD-1 and anti-CTLA-4 antibodies on days 3, 6, and 9. Day 11 and day 21 time points are indicated and represent the time points used to investigate the tumor infiltrating lymphocyte (TIL) landscape. Growth curves were analyzed using area under the curve analysis (AUC). **, $p \leq 0.01$. n=5 for all groups, except for the IgG control timepoint at day 22 (#; n=4).



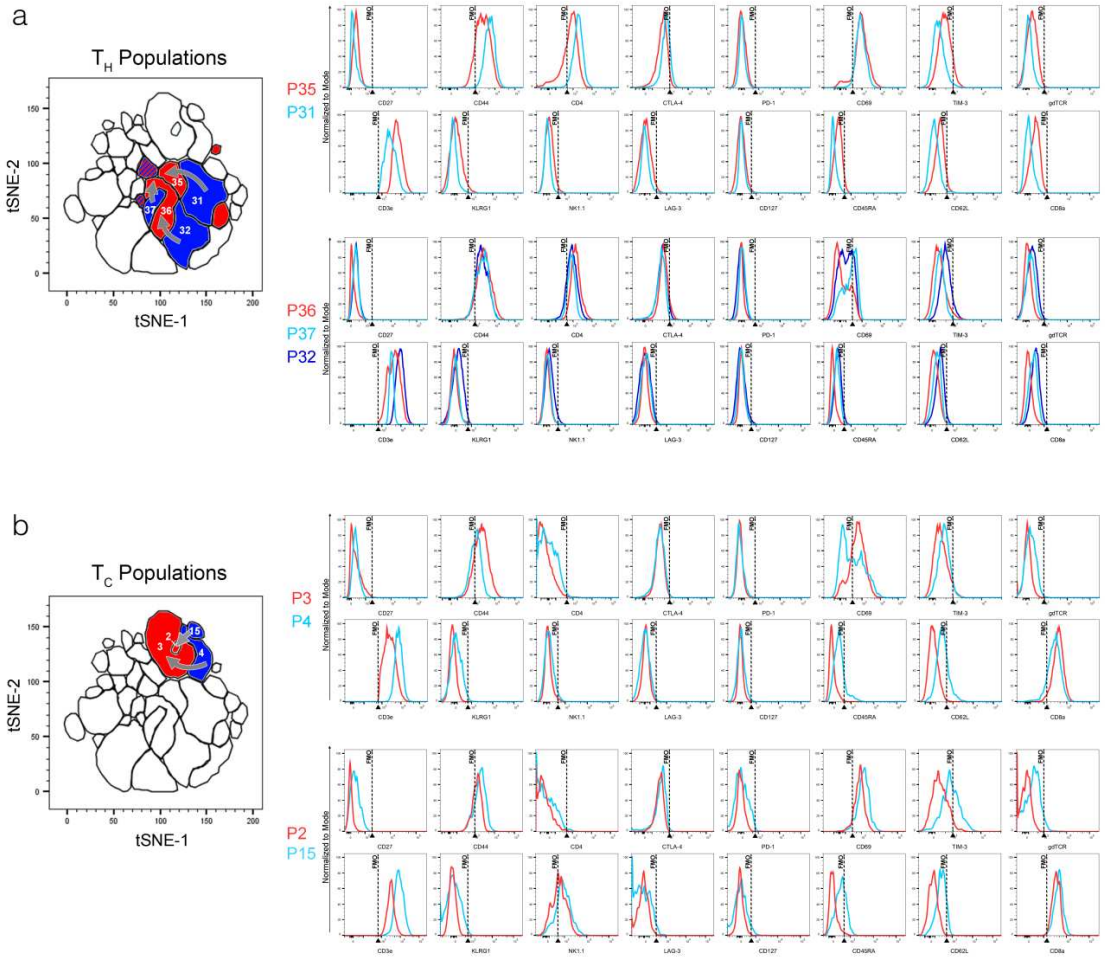
Supplemental Figure 2: Fluorescence-minus-one (FMO) control samples. (a) Small aliquots from each tumor digest were pooled and then stained for all fluorescent parameters except for one as indicated. Cells were first gated on non-aggregates, and then on viable cells before plotting the respective histograms. (b) FMO for Live/Dead Fixable Blue viability dye. Here cells were only gated on non-aggregates prior to plotting side-scatter area parameter versus the channel used for the detection of Live/Dead Fixable Blue.



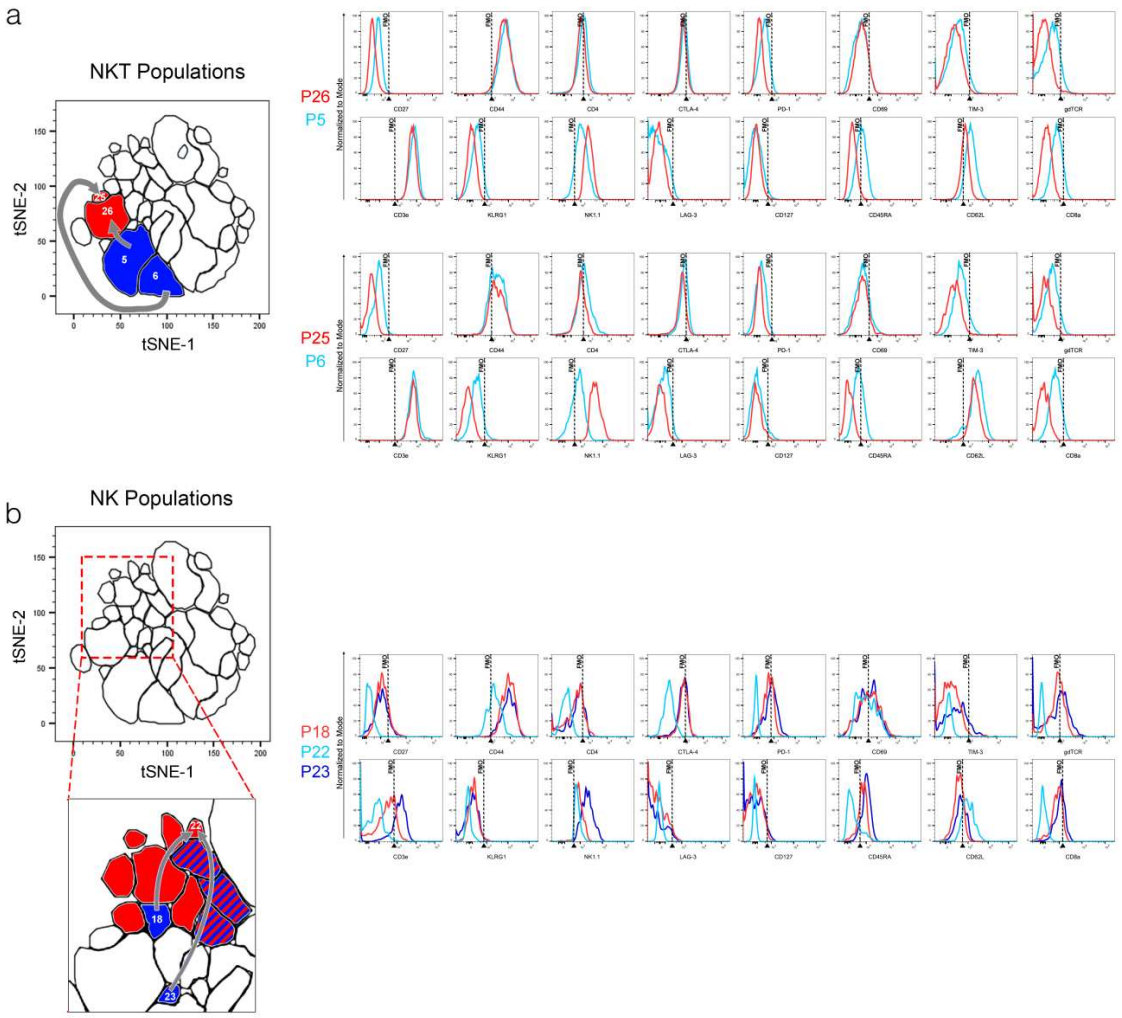
Supplemental Figure 3: Preliminary gating schema. Prior to t-SNE analysis, concatenated data was first gated on non-aggregate events using forward scatter area (FSC-A) versus height (FSC-H) parameters, and then by using side scatter area (SSC-A) versus height (SSC-H) parameters, followed by Live/Dead Fixable Blue-excluding events for viability, followed by SSC-A versus FSC-A parameters to exclude debris, followed by CD3ε and/or NK1.1 positivity.



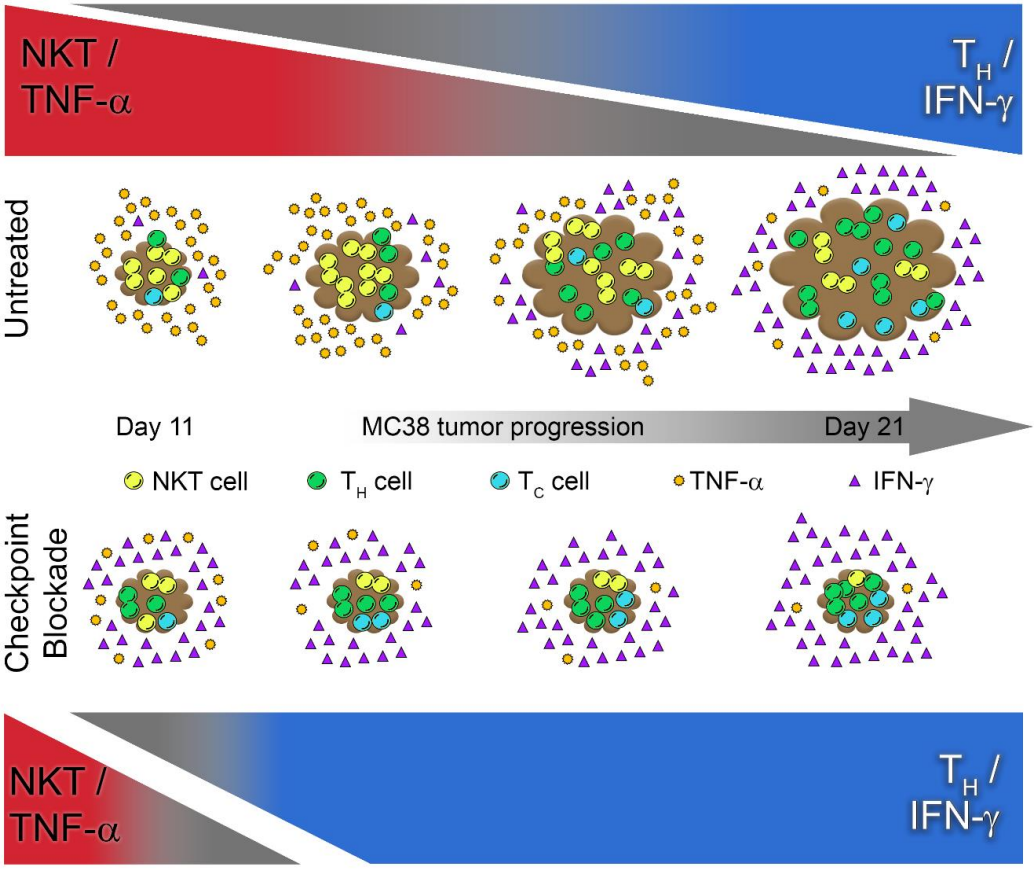
Supplemental Figure 4: t-SNE positive event overlays as determined by FMO controls. Positive and negative events for each indicated parameter were selected using the gates defined by FMO controls as in Supplemental Figure 2, and then overlaid on dot plots of t-SNE parameters. Note that positive events (red) are on top of negative events (blue), and not interspersed.



Supplemental Figure 5: Analogous T cell populations found in TIL isolated from day 11 and day 21 MC38 tumors. (a-b) T_H cell populations (a), and TC populations (b) depicted on the t-SNE map on the left highlighted in blue represent associations with the day 11 time point, while those highlighted in red represent associations with the day 21 time point. Stripped populations were found in both time points. Grey arrows indicate analogous populations closely related to each other from different time points as defined by the hierarchical clustering demonstrated in Figure 1e. Histograms of all clustering parameters are depicted on the right for each set of analogous populations. The MFI at which each FMO gate was set in Supplementary Figure 1 is depicted with a dashed black line.



Supplemental Figure 6: Analogous NKT and NK cell populations found in TIL isolated from day 11 and day 21 MC38 tumors. (a-b) NKT cell populations (a), and NK populations (b) depicted on the t-SNE map on the left highlighted in blue represent associations with the day 11 time point, while those highlighted in red represent associations with the day 21 time point. Stripped populations were found in both time points. Grey arrows indicate analogous populations closely related to each other from different time points as defined by the hierarchical clustering demonstrated in Figure 1e. Histograms of all clustering parameters are depicted on the right for each set of analogous populations. The MFI at which each FMO gate was set in Supplementary Figure 1 is depicted with a dashed black line.



Supplemental Figure 7: A summary schematic of the proposed NKT/TNF-α to T_H/IFN-γ switch. In untreated mice, the anti-tumor immune response is dominated by NKT cells and TNF-α production during early tumor growth as measured 11 days following inoculation. This response subsides and is surpassed by conventional T cell (and in particular T_H cell) responses and IFN-γ production in later tumor growth as measured 21 days following inoculation. In mice treated with checkpoint blockade, this switch occurs more quickly with significantly greater frequencies of T_H cells and IFN-γ production already set in place at day 11 post inoculation.

Supplemental Table 1: Antibody Information							
Antigen	**Conjugation**	**Channel**	**Vendor**	**Cat**	**Clone**	**µl***	**Use****
CD3ε	PE-Cy5	YG-C 670/30	BD Biosciences	553065	145-2C11	2.5	PG, C
CD4	BUV563	UV-E 586/15	BD Biosciences	565709	GK1.5	5	C
CD8a	BUV805	UV-A 820/60	BD Biosciences	564920	53-6.7	1.25	C
CD69	PE-CF594	YG-D 610/20	BD Biosciences	562455	H1.2F3	1	C
CD62L	PE-Cy7	YG-A 780/60	Biolegend	104418	MEL-14	0.5	C
CD27	BV510	Violet -F 525/50	BD Biosciences	563605	LG.3A10	5	C
CD45RA	BV786	Violet-A 780/60	BD Biosciences	564361	14.8	1	C
CD44	AF488	Blue-E 530/30	Biolegend	103016	IM7	0.5	C
KLRG-1	PerCP-Cy5.5	Blue-B 710/50	Biolegend	138418	2F/KLRG1	0.5	C
CD45.2	APC-Cy7	Red-A 780/60	Biolegend	109824	104	1	P
PD-1	BV605	Violet-D 610/20	BD Biosciences	563059	J43	0.5	C
LAG-3	BV711	Violet B- 710/50	BD Biosciences	563179	C9B7W	0.5	C
TIM-3	APC	Red-C 670/30	Biolegend	119706	RMT3-23	2	C
CTLA-4	PE	YG-E 586/15	BD Biosciences	553720	UC10-4F10-11	2	C
CD127	BUV737	UV-B 740/35	BD Biosciences	564399	SB/199	1	C
NK1.1	AF700	Red-B 710/50	BD Biosciences	560515	PK136	10	PG, C
CD107a	BUV395	UV-H 379/28	BD Biosciences	565533	1D4B	10	E
γδTCR	BUV661	UV-C 370/30	BD Biosciences	750766	V65	5	C
INF-g	BV421	Violet G-450/50	BD Biosciences	563376	XMG1.2	5	E
TNFα	BV650	Violet-C 670/30	BD Biosciences	563943	MP6-XT22	5	E
L/D Fix Blue		UV-G 450/50	Invitrogen	L23105	N/A	1	PG
α-GalCer tet.	PE	YG-E 586/15	ProImmune	E001-2A-D	N/A	2	S

Supplemental Table 1: Flow cytometry antibodies

*Optimal µl antibody/dye used per 100 µl staining volume per $1x10^6$ cells as determined by staining index titration.

** PG = preliminary gating; C = clustering; E = effector function; S = supplementary panel

Supplementary Table 2: Comparisons of t-SNE Population Frequencies after Treatment with Checkpoint Blockade								
Population	Day 11				Day 21			
	Iso Tx	α-PD1	α-CTLA4	Combo	Iso Tx	α-PD1	α-CTLA4	Combo
1	0.31 ± 0.12	0.69 ± 0.26	**3.8 ± 1.5****	**3.6 ± 1.2****	**0.03 ± 0.01***	0.27 ± 0.22	0.12 ± 0.07	0.32 ± 0.22
2	0.21 ± 0.13	0.08 ± 0.02	0.10 ± 0.10	0.10 ± 0.00	**2.6 ± 0.41***	**1.4 ± 0.67†**	**0.62 ± 0.28†**	**0.51 ± 0.20†**
3	3.1 ± 0.38	2.7 ± 0.38	2.9 ± 0.36	2.4 ± 0.59	**29 ± 0.32***	25 ± 2.0	12 ± 2.8††	13 ± 2.5†
4	2.2 ± 0.52	4.0 ± 0.54	**4.2 ± 0.44***	**4.5 ± 0.61***	1.9 ± 0.08	2.4 ± 0.46	**0.90 ± 0.23†**	**0.72 ± 0.17†**
5	46 ± 3.7	30 ± 4.3	26 ± 5.9	**11 ± 3.9****	**0.22 ± 0.04***	0.18 ± 0.05	0.19 ± 0.04	0.23 ± 0.08
6	7.5 ± 1.1	8.2 ± 1.2	9.8 ± 1.5	8.8 ± 1.7	**0.29 ± 0.12***	0.32 ± 0.09	0.16 ± 0.06	0.01 ± 0.01
7	0.70 ± 0.41	0.50 ± 0.15	0.42 ± 0.15	0.22 ± 0.10	**0.01 ± 0.01***	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
8	1.3 ± 0.16	2.3 ± 0.53	1.3 ± 0.23	1.3 ± 0.18	0.73 ± 0.18	1.3 ± 0.40	0.88 ± 0.10	0.76 ± 0.18
9	0.59 ± 0.16	1.1 ± 0.29	0.87 ± 0.17	**1.4 ± 0.34***	**0.09 ± 0.02***	**0.04 ± 0.01†**	0.07 ± 0.03	0.08 ± 0.05
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**1.7 ± 0.19***	2.4 ± 0.65	1.3 ± 0.29	2.9 ± 0.77
11	0.9 ± 0.28	0.91 ± 0.13	0.52 ± 0.15	0.65 ± 0.19	**0.04 ± 0.00***	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.02
12	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**2.4 ± 0.61***	2.6 ± 0.61	**0.61 ± 0.14††**	0.62 ± 0.23
13	1.4 ± 0.24	**0.50 ± 0.15***	**0.33 ± 0.04****	**0.14 ± 0.07****	**3.5 ± 0.46***	4.1 ± 0.58	2.5 ± 0.56	2.2 ± 0.72
14	0.42 ± 0.23	0.08 ± 0.02	0.08 ± 0.02	0.16 ± 0.08	0.85 ± 0.46	0.85 ± 0.38	0.80 ± 0.27	0.27 ± 0.08
15	0.75 ± 0.23	1.2 ± 0.28	1.3 ± 0.32	0.69 ± 0.28	0.34 ± 0.07	**0.20 ± 0.01†**	**0.05 ± 0.02††**	**0.07 ± 0.06†**
16	1.0 ± 0.10	0.82 ± 0.14	1.0 ± 0.19	0.89 ± 0.18	**0.33 ± 0.1***	0.23 ± 0.04	0.27 ± 0.06	0.52 ± 0.28
17	0.01 ± 0.07	0.08 ± 0.02	0.04 ± 0.02	0.09 ± 0.04	**10 ± 2.6***	9.4 ± 2.1	**3.3 ± 1.9†**	5.1 ± 2.3
18	1.6 ± 0.28	1.9 ± 0.42	0.96 ± 0.18	0.83 ± 0.19	**0.17 ± 0.04***	0.27 ± 0.07	**0.05 ± 0.03†**	0.21 ± 0.06
19	1.4 ± 0.29	1.2 ± 0.19	0.87 ± 0.14	0.94 ± 0.11	0.57 ± 0.08	1.0 ± 0.22	**1.0 ± 0.08†**	1.3 ± 0.29
20	2.1 ± 0.23	1.7 ± 0.18	1.8 ± 0.13	1.8 ± 0.21	3.0 ± 0.36	**1.3 ± 0.20†**	**1.5 ± 0.16††**	1.6 ± 0.32
21	0.30 ± 0.14	0.52 ± 0.13	0.35 ± 0.08	0.35 ± 0.11	0.54 ± 0.09	0.41 ± 0.08	0.30 ± 0.08	0.54 ± 0.15
22	0.09 ± 0.04	0.01 ± 0.01	**0.00 ± 0.00***	**0.01 ± 0.00***	**0.34 ± 0.05***	**0.64 ± 0.13†**	0.23 ± 0.09	0.17 ± 0.06
23	0.86 ± 0.29	0.84 ± 0.19	0.42 ± 0.05	0.39 ± 0.10	**0.00 ± 0.00***	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
24	0.02 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.01 ± 0.01	**1.8 ± 0.34***	**0.57 ± 0.17†**	1.6 ± 1.2	0.75 ± 0.41
25	0.04 ± 0.04	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.04	**1.2 ± 0.18***	0.92 ± 0.22	1.6 ± 0.28	1.2 ± 0.36
26	0.96 ± 0.30	0.46 ± 0.10	0.35 ± 0.14	**0.09 ± 0.04***	**14 ± 1.5***	12 ± 1.8	29 ± 4.7	11 ± 1.7
27	0.64 ± 0.19	0.32 ± 0.09	0.48 ± 0.15	**0.22 ± 0.06***	**5.2 ± 0.59***	**3.2 ± 0.29††**	6.7 ± 1.3	3.0 ± 0.47
28	0.59 ± 0.16	0.68 ± 0.13	0.51 ± 0.12	0.70 ± 0.14	1.5 ± 0.24	2.1 ± 0.34	**0.85 ± 0.12†**	2.5 ± 0.99
29	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.03	**0.12 ± 0.04***	0.17 ± 0.06	0.10 ± 0.05	0.03 ± 0.02
30	0.07 ± 0.04	0.02 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.07 ± 0.03	0.11 ± 0.04	0.83 ± 0.4	0.04 ± 0.03
31	9.2 ± 2.3	17 ± 2.6	14 ± 2.5	**21 ± 3.1***	**2.5 ± 0.18***	3.4 ± 0.32	**3.5 ± 0.32†**	4.6 ± 0.91
32	5.0 ± 0.90	**12 ± 1.3****	**18 ± 3.5****	**23 ± 2.4****	**0.79 ± 0.09***	1.0 ± 0.14	0.94 ± 0.15	0.87 ± 0.24
33	0.75 ± 0.26	0.81 ± 0.14	**0.25 ± 0.09***	**0.26 ± 0.07***	1.7 ± 0.30	2.3 ± 0.38	2.1 ± 0.36	2.2 ± 0.21
34	1.3 ± 0.14	**3.1 ± 0.28****	**3.6 ± 0.78****	**4.7 ± 0.78****	**0.12 ± 0.04***	0.07 ± 0.02	0.06 ± 0.02	0.24 ± 0.08
35	3.1 ± 0.90	1.3 ± 0.35	**0.61 ± 0.11****	**1.1 ± 0.23***	2.8 ± 0.10	3.8 ± 0.45	**8.4 ± 1.5††**	**18 ± 4.0†**
36	2.9 ± 0.72	2.4 ± 0.50	1.6 ± 0.25	3.1 ± 0.47	**8.7 ± 0.96***	**15 ± 2.4†**	**15 ± 1.4††**	**24 ± 2.9†**
37	2.6 ± 0.34	2.9 ± 0.31	2.8 ± 0.46	**4.6 ± 0.57***	**0.61 ± 0.16***	0.68 ± 0.13	**1.1 ± 0.13†**	0.83 ± 0.06

Supplemental Table 2. Mann Whitney U comparisons of t-SNE Population Frequencies after treatment with checkpoint blockade.

Iso = isotype control

* p<0.05 versus Iso, Day 11

** p<0.01 versus Iso, Day 11

*** p<0.001 versus Iso, Day 11

† p< 0.05 versus Iso, Day 21

†† p< 0.01 versus Iso, Day 21

††† p< 0.001 versus Iso, Day 21

Supplementary Table 3: Linear Regression Analyses of t-SNE Populations and Tumor Volume						
Population	Day 11			Day 21		
	P value	R square	Slope	P value	R square	Slope
1	0.2045	0.0612		0.4501	0.0262	
2	0.8969	0.0007		**0.0063**	**0.2932**	**Positive**
3	0.9952	<0.0001		**0.0204**	**0.2211**	**Positive**
4	**0.0370**	**0.1567**	**Positive**	0.2719	0.0546	
5	**0.0200**	**0.1912**	**Negative**	0.2998	0.0488	
6	0.3355	0.3571		0.8967	0.0008	
7	0.7584	0.0037		0.4335	0.0281	
8	0.8566	0.0013		0.9176	0.0110	
9	0.3197	0.0381		0.2697	0.0551	
10	0.8102	0.0023		0.9300	0.0004	
11	0.1469	0.0792		0.2420	0.0617	
12	**0.0010**	**0.3438**	**Positive**	0.5225	0.0188	
13	**0.0084**	**0.2384**	**Positive**	0.4009	0.0323	
14	0.7562	0.0038		0.7357	0.0053	
15	0.6668	0.0072		**0.0452**	**0.1701**	**Positive**
16	0.8975	0.0170		0.1390	0.0968	
17	0.9893	<0.0001		0.7177	0.0061	
18	0.2488	0.0508		0.4269	0.0289	
19	0.5714	0.0125		0.2864	0.0515	
20	0.3125	0.0392		**0.0001**	**0.4912**	**Positive**
21	0.8528	0.0013		0.2597	0.0573	
22	**0.0037**	**0.2806**	**Positive**	0.6608	0.0089	
23	0.1742	0.0698		0.8779	0.0011	
24	0.4765	0.0197		0.7583	0.0044	
25	0.6538	0.0079		0.7283	0.0056	
26	**0.0278**	**0.1728**	**Positive**	0.4457	0.0267	
27	0.4842	0.0190		0.9214	0.0005	
28	0.7328	0.0046		0.2405	0.0621	
29	0.3239	0.0374		0.8939	0.0008	
30	0.8615	0.0438		0.5611	0.0156	
31	0.2565	0.492		0.0719	0.1398	
32	**0.0443**	**0.1466**	**Negative**	0.7548	0.0045	
33	0.2659	0.0474		0.4943	0.0215	
34	**0.0248**	**0.1791**	**Negative**	0.4319	0.0283	
35	**0.0277**	**0.1729**	**Positive**	0.1347	0.0988	
36	0.6023	0.0106		0.5533	0.0162	
37	0.1272	0.0872		0.7676	0.0041	

Supplemental Table 3: Linear regression analyses of t-SNE populations and tumor volume at excision.

Supplementary Table 4: Comparisons of TNFα-producing Cell Frequencies Among t-SNE Populations after Treatment with Checkpoint Blockade					
Population	Time point	Iso Tx	α-PD1	α-CTLA4	Combo
1	Day 11	82.5 ± 11.81	**24.06 ± 11.77***	**32.19 ± 2.971****	**38.04 ± 4.571****
2	Day 11	62.5 ± 23.94	45.84 ± 15.02	43.75 ± 17.52	28.88 ± 14.33
2	Day 21	63.28 ± 4.668	51.55 ± 10.21	44.25 ± 10.83	33.33 ± 20.41
3	Day 11	10.08 ± 2.381	11.22 ± 4.469	10.4 ± 2.683	10.37 ± 3.884
3	Day 21	17.95 ± 2.497	13.11 ± 5.518	13.22 ± 2.89	7.81 ± 4.959
4	Day 11	8.523 ± 3.17	14.75 ± 2.99	14.99 ± 3.298	9.616 ± 2.639
4	Day 21	15.45 ± 3.826	15.32 ± 5.896	11.71 ± 4.324	19.33 ± 10.73
5	Day 11	99.18 ± 0.2136	97.81 ± 0.4846	97.43 ± 0.7083	**86.88 ± 6.285****
5	Day 21	78.55 ± 9.241	63.41 ± 10.83	67.09 ± 14.23	55.35 ± 21.1
6	Day 11	77.15 ± 4.4	63.24 ± 3.226	**53.46 ± 3.578****	**31.32 ± 8.461****
8	Day 11	12.84 ± 2.944	**6.229 ± 1.36***	7.221 ± 1.448	**3.053 ± 1.093****
9	Day 11	7.65 ± 4.642	6.365 ± 3.524	11.36 ± 2.418	5.884 ± 3.805
10	Day 21	1.685 ± 0.5898	1.888 ± 0.9146	4.116 ± 2.199	1.605 ± 0.9431
11	Day 11	2.5 ± 2.50	2.636 ± 1.585	0.5 ± 0.50	4.909 ± 1.908
12	Day 21	94.73 ± 2.339	94.48 ± 1.722	**99.4 ± 0.60****	75 ± 25
13	Day 11	28.93 ± 4.03	34.51 ± 10.3	46.09 ± 9.541	27.54 ± 12
13	Day 21	10.46 ± 0.8996	6.594 ± 3.092	**2.176 ± 1.088****	**2.505 ± 0.9513***
14	Day 21	10.05 ± 7.919	10.99 ± 6.521	4.234 ± 2.277	2.273 ± 2.273
15	Day 11	95.18 ± 3.371	77.86 ± 8.405	77.56 ± 8.266	**48.84 ± 15.25***
16	Day 11	40.02 ± 12.03	32.39 ± 7.499	31.11 ± 3.389	14.87 ± 4.853
17	Day 21	37.05 ± 3.152	54.41 ± 6.128	52.21 ± 8.23	42.73 ± 13.05
18	Day 11	98.45 ± 1.55	99.56 ± 0.2903	94.19 ± 2.994	93.26 ± 6.185
18	Day 21	44.93 ± 17.7	67.48 ± 11.02	37.5 ± 15.67	81.25 ± 11.97
19	Day 11	21 ± 5.916	12.43 ± 2.676	12.98 ± 4.024	**7.22 ± 2.974***
19	Day 21	7.135 ± 0.9225	8.284 ± 2.976	6.296 ± 1.522	3.575 ± 3.575
20	Day 11	14.09 ± 7.649	8.061 ± 1.833	10.34 ± 3.49	6.724 ± 1.923
20	Day 21	44.9 ± 3.774	18.07 ± 6.586	**12.98 ± 4.524****	**8.533 ± 6.081***
24	Day 21	21.58 ± 4.41	25.37 ± 6.351	**12.44 ± 7.025***	16.93 ± 5.733
25	Day 21	73.2 ± 5.361	43.05 ± 9.126	60.28 ± 8.415	**28.33 ± 6.453***
26	Day 21	92.75 ± 2.069	92.64 ± 0.8313	90.21 ± 2.026	88.13 ± 2.008
27	Day 21	78.45 ± 4.414	76.28 ± 3.918	74.86 ± 5.37	82.4 ± 3.848
28	Day 11	60.3 ± 7.355	67.33 ± 3.601	65.19 ± 8.016	**34 ± 7.255***
28	Day 21	41.4 ± 3.277	48.25 ± 4.438	42.29 ± 4.881	45.98 ± 12.39
31	Day 11	12.02 ± 1.705	**6.711 ± 1.123***	**6.543 ± 0.786***	**3.956 ± 1.247****
31	Day 21	1.27 ± 1.27	1.279 ± 0.5701	1.729 ± 0.7084	0.3625 ± 0.3625
32	Day 11	9.608 ± 3.05	5.888 ± 1.183	6.268 ± 1.121	**3.686 ± 0.6455***
34	Day 11	11.82 ± 5.831	7.984 ± 1.455	8.478 ± 2.369	3.28 ± 1.072
35	Day 11	8.265 ± 1.866	4.878 ± 2.114	4.458 ± 1.723	10.42 ± 3.719
35	Day 21	3.113 ± 0.8758	2.633 ± 1.061	4.948 ± 0.8484	2.745 ± 1.416
36	Day 11	8.013 ± 1.009	4.581 ± 1.338	3.751 ± 1.544	**3.736 ± 0.6016***
36	Day 21	1.235 ± 0.4726	2.164 ± 1.077	1.989 ± 0.6741	1.41 ± 0.7383
37	Day 11	6.35 ± 3.724	6.069 ± 1.34	4.278 ± 1.438	2.47 ± 0.5724

Supplemental Table 4. Comparison of the percentages of cells staining positive for TNFα among t-SNE Populations after checkpoint blockade. Shown are group means plus/minus standard error. Asterisks and dagger symbols indicate a significant difference versus isotype control antibody-treated mice (Iso Tx) at Day 11 or Day 21 of MC38 tumor growth respectively by Mann-Whitney test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Note: Not all populations contained cells at both time points or across all treatments, and some populations did not have enough $TNF\alpha^+$ cells to analyze.

Supplementary Table 5: Comparisons of TNFα Median Fluorescent Intensities Among t-SNE Populations after Treatment with Checkpoint Blockade					
Population	Time point	Iso Tx	α-PD1	α-CTLA4	Combo
1	Day 11	6041 ± 2609	3874 ± 864.4	**3189 ± 516.7***	**2455 ± 214.7***
2	Day 11	17160 ± 1323	18550 ± 6921	11850 ± 2326	12270 ± 1811
2	Day 21	4949 ± 978.2	4957 ± 556.9	4065 ± 407.6	3618 ± 1425
3	Day 11	3685 ± 350.9	4412 ± 665.2	3831 ± 767.6	4413 ± 1233
3	Day 21	3858 ± 313.2	4310 ± 556.8	3367 ± 179.5	3199 ± 346
4	Day 11	9639 ± 2937	**4202 ± 653.8***	**4207 ± 900.5***	**3019 ± 384.8****
4	Day 21	3217 ± 351	2825 ± 447.2	4527 ± 908.2	7884 ± 4212
5	Day 11	16060 ± 1259	14500 ± 970.1	14520 ± 890.4	**9688 ± 974.6****
5	Day 21	5702 ± 1386	5426 ± 1078	5313 ± 1016	3593 ± 783
6	Day 11	10680 ± 882.9	**8038 ± 347.1***	**6968 ± 610.1***	**5116 ± 867.5****
7	Day 11	39560 ± 8395	35710 ± 4394	29760 ± 4056	37900 ± 9447
8	Day 11	8058 ± 2317	4278 ± 1168	3646 ± 591.8	3358 ± 954.3
12	Day 21	60120 ± 2822	56820 ± 4757	**45600 ± 6823***	46590 ± 5862
13	Day 11	15160 ± 2934	12280 ± 1147	10410 ± 1744	10260 ± 506.9
13	Day 21	7703 ± 2110	6707 ± 1710	7848 ± 2472	5382 ± 1179
15	Day 11	14750 ± 2158	17660 ± 3522	16100 ± 4556	10060 ± 2099
16	Day 11	6782 ± 1573	5480 ± 1000	8138 ± 1251	4758 ± 778.7
17	Day 21	13050 ± 1966	10090 ± 3269	11300 ± 3071	14810 ± 6294
18	Day 11	20830 ± 2861	20980 ± 1072	19020 ± 391.3	17550 ± 1372
18	Day 21	10650 ± 1634	13660 ± 6266	16960 ± 7646	10750 ± 4068
19	Day 11	9640 ± 3231	12620 ± 3717	5561 ± 1307	7479 ± 1666
20	Day 11	2482 ± 258.4	3533 ± 580	**4802 ± 1057***	4116 ± 828.4
20	Day 21	5033 ± 919.1	6327 ± 1986	4836 ± 1384	4100 ± 137.5
23	Day 11	40410 ± 4930	41550 ± 3239	41230 ± 5415	44810 ± 3584
24	Day 21	3938 ± 582.3	11100 ± 6499	4188 ± 928.4	12710 ± 5501
25	Day 21	4632 ± 540.7	4294 ± 440.9	3912 ± 284.7	3623 ± 764.5
26	Day 11	21410 ± 1950	22490 ± 648.6	19900 ± 1495	17660 ± 2082
26	Day 21	6462 ± 792	6263 ± 530.4	4934 ± 546.3	4673 ± 404.4
27	Day 11	10980 ± 2067	9371 ± 1696	10800 ± 1207	6903 ± 1006
27	Day 21	4232 ± 339.4	4777 ± 350	4033 ± 277.4	4291 ± 747.3
28	Day 11	12910 ± 3689	7371 ± 1687	8840 ± 1830	5118 ± 660.9
28	Day 21	8018 ± 560.6	5953 ± 812.8	6338 ± 1012	4938 ± 801
31	Day 11	3981 ± 423.5	**2780 ± 176.4***	3118 ± 252.6	2832 ± 473.2
32	Day 11	4164 ± 476.6	**2810 ± 126.9****	4530 ± 1708	**3141 ± 357.6***
34	Day 11	5005 ± 556.3	**3037 ± 315.7***	2932 ± 480.3	3491 ± 490.9
35	Day 11	1988 ± 22.1	2300 ± 186.8	2305 ± 317	3098 ± 635.7
35	Day 21	2526 ± 554.6	3132 ± 626	3046 ± 284.2	2340 ± 302.6
36	Day 11	3980 ± 730.6	**2277 ± 134.4***	3166 ± 463.3	3050 ± 340.7
36	Day 21	2907 ± 693.5	3270 ± 394.3	3334 ± 419.7	2600 ± 40.59
37	Day 11	6345 ± 1974	4843 ± 1127	3165 ± 426.3	3338 ± 621.3

Supplemental Table 5. Comparison of TNFα median fluorescent intensities (MFI) among t-SNE Populations after treatment with checkpoint inhibitors checkpoint blockade. Shown are group means plus/minus standard error. Asterisks and dagger symbols indicate a significant difference versus isotype control antibody-treated mice (Iso Tx) at Day 11 or Day 21 of MC38 tumor growth respectively by Mann-Whitney test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Note: Not all populations contained cells at both time points or across all treatments, and some populations did not have enough $TNF\alpha^+$ cells to analyze.

Supplementary Table 6: Comparisons of IFN-γ-producing Cell Frequencies Among t-SNE Populations after Treatment with Checkpoint Blockade

Population	Time point	Iso Tx	α-PD1	α-CTLA4	Combo
1	Day 11	75 ± 18.93	44.11 ± 16.06	69.09 ± 7.088	59.18 ± 8.883
2	Day 11	6.25 ± 6.25	6.25 ± 6.25	7.638 ± 6.206	16.06 ± 10.6
2	Day 21	23.38 ± 5.563	16.78 ± 3.992	18.73 ± 6.478	6.25 ± 6.25
3	Day 11	31.13 ± 4.611	29.48 ± 5.194	43.95 ± 7.943	54.25 ± 7.585
3	Day 21	20.15 ± 1.189	14.79 ± 1.663	22.31 ± 3.64	16.09 ± 3.799
4	Day 11	24.48 ± 5.37	20.85 ± 3.029	39.79 ± 7.176	**51.56 ± 6.392***
4	Day 21	16.73 ± 2.219	11.48 ± 3.831	13.78 ± 3.653	19.33 ± 10.73
5	Day 11	75.65 ± 6.325	**57.94 ± 4.851***	70.04 ± 4.541	75.93 ± 5.748
5	Day 21	73.35 ± 9.023	69.38 ± 11.15	51.26 ± 12.26	55.35 ± 21.1
6	Day 11	59.08 ± 9.407	54.51 ± 4.918	64.56 ± 5.75	67.23 ± 5.883
8	Day 11	2.918 ± 1.719	6.429 ± 2.052	20.74 ± 6.546	**44.46 ± 9.703****
9	Day 11	10.85 ± 5.365	6.419 ± 2.754	17.09 ± 8.032	19.59 ± 6.71
10	Day 21	0.675 ± 0.675	0.0675 ± 0.0675	0.625 ± 0.4126	0.455 ± 0.455
12	Day 21	96.9 ± 0.7627	96.98 ± 1.989	98.15 ± 1.307	98.33 ± 1.675
13	Day 11	19.87 ± 4.653	35.25 ± 10.66	45.42 ± 11.83	26.43 ± 10.83
13	Day 21	13.99 ± 2.314	9.101 ± 3.247	**3.485 ± 1.163****	5.185 ± 3.117
14	Day 11	45.25 ± 23.21	41.08 ± 14.83	37.8 ± 15.16	39.39 ± 13.62
14	Day 21	17.48 ± 7.487	28.79 ± 5.475	**59.45 ± 11.43***	59.1 ± 24.75
15	Day 11	46.53 ± 19.02	19.14 ± 4.548	35.33 ± 6.969	54.59 ± 11.88
16	Day 11	57.33 ± 5.132	59.23 ± 8.41	65.76 ± 9.285	60.4 ± 10.14
16	Day 21	53.63 ± 10.22	26.95 ± 9.101	**20.79 ± 7.835***	**8.35 ± 4.821***
17	Day 21	78.45 ± 6.478	80.95 ± 2.019	92.74 ± 2.766	81.05 ± 2.602
18	Day 11	92.13 ± 4.256	87.51 ± 4.44	91.1 ± 2.897	76.96 ± 12.7
19	Day 11	12.22 ± 5.203	14.15 ± 2.872	**36.35 ± 6.949***	49.33 ± 12.25
19	Day 21	12.69 ± 4.261	11.55 ± 3.532	7.781 ± 2.015	7.06 ± 4.973
20	Day 11	29.9 ± 7.788	26.71 ± 2.26	43.23 ± 5.382	45.65 ± 8.916
20	Day 21	79.23 ± 1.867	46.54 ± 12.24	**29.31 ± 5.401****	**14.27 ± 7.552***
23	Day 11	70.98 ± 13.97	57.86 ± 5.771	75.44 ± 7.143	69.51 ± 11.73
24	Day 21	50.3 ± 3.097	**70.79 ± 7.481***	51.91 ± 9.312	50.58 ± 20.42
25	Day 21	53.68 ± 9.69	49.23 ± 7.684	52.96 ± 7.805	34 ± 14.47
26	Day 11	63.63 ± 9.155	61.9 ± 8.54	59.75 ± 11.79	27.15 ± 10.95
26	Day 21	87.08 ± 3.759	75.53 ± 5.713	73.2 ± 4.36	70.18 ± 11.32
27	Day 11	72.75 ± 9.795	81.25 ± 6.632	68 ± 6.118	74.6 ± 11.93
27	Day 21	91.85 ± 0.4113	91.6 ± 2.022	**82.2 ± 6.075***	93.83 ± 2.222
28	Day 11	62.93 ± 5.289	61.56 ± 8.53	70.35 ± 7.609	68.75 ± 11.77
28	Day 21	70.7 ± 4.009	72.99 ± 2.905	77.43 ± 4.523	71.6 ± 7.929
29	Day 21	8.325 ± 8.325	17.56 ± 6.316	15.63 ± 12.44	25 ± 25
30	Day 11	50 ± 28.87	18.75 ± 13.15	62.5 ± 18.3	18.75 ± 13.15
30	Day 21	45.83 ± 20.84	59.94 ± 12.93	55.55 ± 13.77	25 ± 25
31	Day 11	8.93 ± 2.666	14.26 ± 3.09	**29.05 ± 7.616***	**44.51 ± 5.531****
31	Day 21	1.94 ± 1.027	1.991 ± 0.7307	2.891 ± 0.8262	4.44 ± 2.612
32	Day 11	9.815 ± 1.695	13.82 ± 3.305	**31.01 ± 7.32***	**45.98 ± 5.679****
32	Day 21	7.043 ± 3.105	1.539 ± 0.8301	12.23 ± 2.487	15.89 ± 11.49
34	Day 11	10.19 ± 5.298	7.546 ± 2.251	26.33 ± 8.606	**47.28 ± 6.361****
35	Day 11	17.6 ± 1.439	13.65 ± 4.392	**26.77 ± 9.114***	**38.55 ± 7.338****
35	Day 21	3.718 ± 0.5425	4.945 ± 0.9764	5.065 ± 1.13	7.708 ± 2.308
36	Day 11	7.935 ± 3.859	12.35 ± 4.265	29.48 ± 6.779	**42.39 ± 6.818****
36	Day 21	5.955 ± 0.4786	5.423 ± 0.8202	5.905 ± 0.9849	5.408 ± 0.7515
37	Day 11	11.73 ± 5.44	20.75 ± 3.813	34.58 ± 8.221	**42.24 ± 5.672****

Supplemental Table 6. Comparison of the percentages of cells staining positive for IFN-γ among t-SNE Populations after checkpoint blockade. Shown are group means plus/minus standard error. Asterisks and dagger symbols indicate a significant difference versus isotype control antibody-treated mice (Iso

Tx) at Day 11 or Day 21 of MC38 tumor growth respectively by Mann-Whitney test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Note: Not all populations contained cells at both time points or across all treatments, and some populations did not have enough IFN-γ^+ cells to analyze.

Table 7: Comparisons of IFN-γ Median Fluorescent Intensities Among t-SNE Populations after Treatment with Checkpoint Blockade

Population	Time point	Iso Tx	α-PD1	α-CTLA4	Combo
1	Day 11	747.3 ± 93.07	467 ± 68.11	561.4 ± 63.51	615.5 ± 27.11
3	Day 11	461.5 ± 61.65	420 ± 33.86	418 ± 38.07	391.6 ± 34.14
3	Day 21	326.3 ± 5.17	380.3 ± 26.99	443.5 ± 50.65	**409.3 ± 31.12***
4	Day 11	424.3 ± 46.69	389.8 ± 31.34	398.4 ± 8.99	369.5 ± 18.13
4	Day 21	336 ± 23.71	388.4 ± 65.59	376.6 ± 26.58	447.3 ± 170.5
5	Day 11	571 ± 32.28	489.3 ± 30.06	568.4 ± 80.65	581.3 ± 45.16
5	Day 21	586 ± 171.1	590.7 ± 53.75	483.3 ± 51.72	450.7 ± 79
6	Day 11	395 ± 23.62	420.6 ± 21.95	501.3 ± 79.01	494.4 ± 42.72
8	Day 11	332.5 ± 79.5	337.7 ± 49.24	308.7 ± 25.34	327.9 ± 7.453
9	Day 11	343 ± 85.5	396 ± 87.27	308.5 ± 19.73	362.7 ± 21.21
12	Day 21	1464 ± 50.19	1190 ± 155.8	**850.5 ± 99.81****	**780.5 ± 201.8***
13	Day 11	552 ± 151.5	941.1 ± 384.4	484.6 ± 93.43	655.3 ± 146.6
14	Day 11	388.3 ± 68.2	428.2 ± 91.91	581.5 ± 147.9	594.4 ± 108
14	Day 21	409.7 ± 101.6	447.4 ± 85.43	388 ± 27.59	359.7 ± 66.12
15	Day 11	635.3 ± 304.9	406.1 ± 19.82	501 ± 54.3	436.3 ± 20.16
16	Day 11	735.8 ± 219.6	1100 ± 238.1	967.5 ± 129.7	1293 ± 362.8
16	Day 21	777 ± 138.7	759.4 ± 111.3	586.6 ± 107.6	590 ± 220
17	Day 21	536 ± 40.81	531.4 ± 61.65	**658.9 ± 32.42***	566 ± 103.6
18	Day 11	711.8 ± 87	640.9 ± 52.58	774.6 ± 90.67	817 ± 104
18	Day 21	701.3 ± 76.14	879.1 ± 147.3	723.5 ± 179.9	583.3 ± 73.9
19	Day 11	345.7 ± 56.33	599.1 ± 184.8	524 ± 132.7	429.1 ± 28.67
19	Day 21	515.8 ± 109.8	607.3 ± 138.6	356.5 ± 30.65	390 ± 144
20	Day 11	729.5 ± 304.7	918 ± 290	443.4 ± 17.15	447.8 ± 34.86
20	Day 21	697.5 ± 60.21	624.9 ± 77.68	**397.4 ± 30.31****	448.3 ± 46.48
23	Day 11	517.5 ± 102.4	677 ± 52.18	771.5 ± 69.23	786.4 ± 73.27
24	Day 21	388.8 ± 24.7	429 ± 53.21	426.6 ± 44.47	419 ± 91.76
25	Day 21	402.8 ± 16.84	420 ± 20.2	362.6 ± 16.01	350.7 ± 42.53
26	Day 11	551.5 ± 50.07	461.4 ± 69.54	450.1 ± 77.38	618.5 ± 95.96
26	Day 21	473.3 ± 25.98	426.4 ± 20.11	**391.1 ± 20.03***	411.8 ± 45.48
27	Day 11	485.5 ± 56.87	538.6 ± 61.08	513.3 ± 75.2	663.4 ± 79.62
27	Day 21	550.8 ± 23.24	495.1 ± 25.63	**463 ± 15.1***	464 ± 43.13
28	Day 11	455.5 ± 33.57	426.6 ± 56.56	560.3 ± 61.63	**686.6 ± 77.46***
28	Day 21	492 ± 30.02	532.3 ± 43.51	541.3 ± 104.4	488.8 ± 43.44
30	Day 11	385.5 ± 63.5	434 ± 167	684.8 ± 164.8	555.5 ± 42.5
31	Day 11	283.5 ± 10.04	**440.4 ± 22.63****	**360.3 ± 15.6****	**348.5 ± 13.44***
31	Day 21	439.3 ± 78.23	292 ± 17.36	525.2 ± 179.6	277.3 ± 21.4
32	Day 11	394.5 ± 49.64	364.5 ± 16.38	367 ± 21.05	354.4 ± 8.627
32	Day 21	248.7 ± 5.175	340 ± 45.8	318.9 ± 28.58	675.3 ± 59.6
34	Day 11	608.7 ± 220.4	398.9 ± 40.06	375.1 ± 19.81	370 ± 11.48
35	Day 11	334.3 ± 36.17	408.8 ± 27.41	594.8 ± 249.8	559.1 ± 152.3
35	Day 21	408.8 ± 56.06	604.6 ± 223.5	385 ± 62.76	429 ± 51.3
36	Day 11	295 ± 20.84	343.7 ± 21.71	312.6 ± 14.01	**436.5 ± 93.2***
36	Day 21	280 ± 7.948	**360.1 ± 13.56****	**339 ± 14.9***	**412.3 ± 47.53***
37	Day 11	355.5 ± 64.4	359.9 ± 23.63	324.3 ± 17.4	328.8 ± 12.03

Supplemental Table 7. Comparison of IFN-γ median fluorescent intensities (MFI) among t-SNE Populations after treatment with checkpoint blockade. Shown are group means plus/minus standard error. Asterisks and dagger symbols indicate a significant difference versus isotype control antibody-treated mice (Iso Tx) at Day 11 or Day 21 of MC38 tumor growth respectively by Mann-Whitney test. *, p<0.05; **, p<0.01; ***, p<0.001. Note: Not all populations contained cells at both time points or across all treatments, and some populations did not have enough IFN-γ^+ cells to analyze.

Table 8: Comparisons of Cell-surface CD107a-expressing Cell Frequencies Among t-SNE Populations after Treatment with Checkpoint Blockade					
Population	Time point	Iso Tx	α-PD1	α-CTLA4	Combo
1	Day 11	97.5 ± 2.5	73.78 ± 11.78	**85.86 ± 2.598***	**85.86 ± 2.101***
3	Day 21	0.2875 ± 0.1058	0.6818 ± 0.3231	0.36 ± 0.1533	0.535 ± 0.3993
5	Day 11	83.8 ± 1.536	86.26 ± 1.475	**89.13 ± 1.201***	90.34 ± 3.272
5	Day 21	85.83 ± 9.466	75.05 ± 11.84	74.18 ± 12.31	61.6 ± 21.5
6	Day 11	82.35 ± 3.003	83.93 ± 3.087	82.44 ± 2.836	62.81 ± 6.592
12	Day 21	54.2 ± 6.079	41.76 ± 9.074	73.16 ± 6.865	48.45 ± 18
13	Day 21	12.37 ± 2.975	8.748 ± 3.418	7.189 ± 2.657	**2.315 ± 1.337***
16	Day 11	30.48 ± 7.681	29.08 ± 6.863	19.27 ± 4.82	35.01 ± 10.59
16	Day 21	47.5 ± 5.907	16.39 ± 5.81	**18.6 ± 5.573***	**16.68 ± 11.79***
17	Day 21	72.73 ± 5.263	64.69 ± 4.446	88.19 ± 4.253	69.58 ± 6.647
18	Day 11	49.2 ± 3.848	52.09 ± 6.006	64.59 ± 6.9	60.83 ± 10.07
19	Day 11	1.725 ± 0.9971	3.056 ± 1.37	8.176 ± 3.996	3.525 ± 1.243
19	Day 21	16.57 ± 4.438	9.416 ± 3.566	7.498 ± 2.454	2.273 ± 2.273
20	Day 11	5.318 ± 1.037	7.68 ± 2.065	8.778 ± 1.747	7.713 ± 2.343
20	Day 21	13.68 ± 1.27	26.89 ± 10.69	21.73 ± 5.847	16.02 ± 3.612
23	Day 11	49.15 ± 17.31	61.65 ± 5.508	62.59 ± 4.296	68.29 ± 11.37
24	Day 21	98.38 ± 0.956	97.48 ± 1.352	97.45 ± 2.493	98.88 ± 1.125
25	Day 21	99.28 ± 0.725	85.05 ± 12.17	98.56 ± 0.6333	95.25 ± 2.056
26	Day 11	61.73 ± 12.68	60.4 ± 11.01	59.14 ± 13.79	31.78 ± 12.7
26	Day 21	98.48 ± 0.34	98.35 ± 0.3322	98.95 ± 0.2104	99.55 ± 0.2872
27	Day 11	57.1 ± 14.81	52.04 ± 12.43	69.56 ± 2.216	59.86 ± 14.5
27	Day 21	90.38 ± 2.512	91.24 ± 1.997	96.2 ± 1.085	93.83 ± 2.752
28	Day 11	51.45 ± 9.797	38.76 ± 7.448	48.78 ± 11.09	57.05 ± 10.87
28	Day 21	74.9 ± 6.161	67.26 ± 5.775	64.39 ± 7.383	69.15 ± 10.45
30	Day 21	45.83 ± 20.84	31.19 ± 13.35	42.93 ± 13.42	25 ± 25

Supplemental Table 8. Comparison of the percentages of cells staining positive for cell-surface CD107a among t-SNE Populations after treatment with checkpoint blockade. Shown are group means plus/minus standard error. Asterisks and dagger symbols indicate a significant difference versus isotype control antibody-treated mice (Iso Tx) at Day 11 or Day 21 of MC38 tumor growth respectively by Mann-Whitney test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Note: Not all populations contained cells at both time points or across all treatments, and some populations did not have enough cell-surface $CD107a^+$ cells to analyze.

Table 9: Comparisons of cell-surface CD107a Median Fluorescent Intensities Among t-SNE Populations after Treatment with Checkpoint Blockade

Population	Time point	Iso Tx	α-PD1	α-CTLA4	Combo
1	Day 11	1469 ± 278.8	1909 ± 108.8	**2073 ± 145.6***	1943 ± 139.4
3	Day 21	753.7 ± 56.53	776.1 ± 44.91	1453 ± 534	671.5 ± 39.5
5	Day 11	1895 ± 78.56	**2220 ± 68.75****	**2264 ± 72.73***	**3036 ± 485.8***
5	Day 21	3163 ± 790.3	2076 ± 336.9	2199 ± 346.9	3822 ± 881.4
6	Day 11	3127 ± 438	3759 ± 176.2	3652 ± 231.2	2762 ± 533.3
12	Day 21	1231 ± 71.66	1338 ± 208.8	**1798 ± 146.8****	1261 ± 346
16	Day 11	1704 ± 422.8	1795 ± 354.8	1478 ± 286.2	1681 ± 342.5
16	Day 21	1401 ± 386.5	1073 ± 103.2	1348 ± 351.5	1334 ± 329
17	Day 21	1269 ± 122	1351 ± 192.5	1988 ± 268.6	1664 ± 537.3
18	Day 11	1190 ± 143.4	1565 ± 188.7	1855 ± 325.3	**2423 ± 580.8****
18	Day 21	2310 ± 841.9	2111 ± 314.6	3657 ± 1777	3031 ± 1187
20	Day 11	892.3 ± 170.8	1535 ± 716.6	1375 ± 271	1318 ± 110.2
20	Day 21	767.8 ± 38.53	1296 ± 283	1120 ± 122.4	**1856 ± 472.9***
23	Day 11	1248 ± 144.1	1758 ± 166.3	1795 ± 266.4	**2179 ± 194.5***
24	Day 21	3691 ± 269.2	2868 ± 473	4167 ± 458.7	2779 ± 544.2
25	Day 21	3156 ± 174.8	2751 ± 113.6	2829 ± 251.2	2382 ± 319.1
26	Day 11	1780 ± 282.1	1796 ± 461.8	2049 ± 712.3	1414 ± 474.4
26	Day 21	2255 ± 147.4	2362 ± 228.3	2330 ± 121.7	**2944 ± 303.1***
27	Day 11	1125 ± 165.7	1167 ± 133.6	**2688 ± 734.2***	**2673 ± 777.6***
27	Day 21	1704 ± 27.82	1843 ± 108.8	1758 ± 83.18	1992 ± 246.1
28	Day 11	2929 ± 940.5	1861 ± 315.5	2409 ± 954.4	2126 ± 354.5
28	Day 21	1635 ± 115.7	1481 ± 128.4	2038 ± 252.2	1097 ± 207.1

Supplemental Table 9. Comparison of cell-surface CD107a median fluorescent intensities (MFI) among t-SNE Populations after treatment with checkpoint blockade. Shown are group means plus/minus standard error. Asterisks and dagger symbols indicate a significant difference versus isotype control antibody-treated mice (Iso Tx) at Day 11 or Day 21 of MC38 tumor growth respectively by Mann-Whitney test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Note: Not all populations contained cells at both time points or across all treatments, and some populations did not have enough cell-surface $CD107a^+$ cells to analyze.